Quality changes of pasteurised mango juice during storage.

Part I: Selecting shelf-life markers by integration of a targeted and untargeted multivariate approach

*Scheling Wibowo, Tara Grauwet, Getnet Belete Gedefa, Marc Hendrickx, Ann Van Loey**

Authors are affiliated to:

Laboratory of Food Technology

Leuven Food Science and Nutrition Research Center (LFoRCe)

Department of Microbial and Molecular Systems (M²S)

Katholieke Universiteit Leuven

Kasteelpark Arenberg 22 box 2457

3001 Heverlee

Belgium

* Corresponding author

Fax: +32-16-32.19.60

Telephone: +32-16-32.15.67

E-mail: *ann.vanloey@biw.kuleuven.be*

https://www.biw.kuleuven.be/m2s/clmt/lmt/

Abstract

For the first time, a multivariate approach combining targeted and untargeted data was used to obtain insight into quality changes in pasteurised mango juice (cv. 'Totapuri') as a function of storage (42 °C for 8 weeks). Mango juice samples were formulated with addition of different potential precursors for different quality-related chemical reactions: ascorbic acid, citric acid and sugars. Control (diluted mango puree with water), ascorbic acid-enriched (AA_{250} and AA_{500}), citric acid-enriched (CA, $CA+AA_{250}$ and $CA+AA_{500}$) and sugar-enriched (S) samples were characterised for a range of targeted quality parameters as well as for a volatile fingerprint (untargeted). Selection of shelf-life markers or quality parameters significantly changing during shelf-life was performed over all formulations as well as per mango juice formulation. Our study showed that a common trend over all formulations was observed for colour values (VID > $|0.90|$), while specific shelf-life markers were selected for each formulation. In acidified mango juice samples (CA, $CA+AA_{250}$, $CA+AA_{500}$), more terpene oxides were selected compared to other formulations. In ascorbic acid-enriched samples (AA_{250}, AA_{500}, $CA+AA_{250}$, $CA+AA_{500}$), furfural and ascorbic acid were significantly changing during shelf-life. It seems that the reaction pathways for compounds being formed or degraded upon shelf-life are clearly affected by the acidity level.

Keywords

Mango juice; Multivariate data analysis; Fingerprinting; Shelf-life; Targeted quality parameter; Volatiles

1 INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits produced in the world. According to Food and Agriculture Organization (FAO), Asia is the largest mango producer, in which India contributing for more than 35% of the world‘s production (FAOSTAT, 2012). Several hundreds mango cultivars exist but only a few are commercialised. Cultivars such as ‘Alphonso’, ‘Haden’, ‘Keitt’, ‘Kent’, ‘Tommy Atkins’ and ‘Totapuri’ are known by the consumers and they vary in colour and flavour characteristics (Singh, Singh, Sane, & Nath, 2013). As a seasonal and highly perishable commodity, mango fruit is commonly processed into different products to extend its shelf-life. By applying conventional thermal treatment, such as pasteurisation, a high-acid food product ($pH < 4.6$) is microbiologically safe, thus can be stored at ambient temperature for a long period (Rawson et al., 2011; Silva & Gibbs, 2004). A number of thermally-treated mango products which are available in the market are mango jam, puree, nectar, juice and canned slices (Siddiq, Akhtar, & Siddiq, 2012).

However, even after processing with the aim of shelf-life extension, it is known that food product quality is not constant, or in other words, it changes continuously over time. Even for a shelf-stable product, there is a limitation in its shelf-life due to deteriorative chemical reactions determining its best-before date (Robertson, 1999). Among all quality attributes, colour is often the first characteristic consumers use to evaluate the overall product quality. Colour can indeed be used as an indicator for a range of different (bio)chemical reactions (van Boekel, 2009). Besides colour, volatile compounds can also be linked to a broad scale of chemical reactions occurring during processing and storage (Kebede et al., 2015; Perez-Cacho & Rouseff, 2008). According to several studies, different fruit juices showed a distinct rate of quality degradation during storage (Polydera, Stoforos, & Taoukis, 2005; Vásquez-Caicedo,

Schilling, & Carle, 2007; Burdurlu & Karadeniz, 2003; Aguiló-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009; Burdurlu et al., 2003). Consequently, it is clear that fruit juice composition plays an important role in fruit juice stability.

In evaluating food quality changes, two approaches are described in literature (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014). The 'targeted analytical approach' is a hypothesis-driven approach. In this approach, one or more food characteristics of interest are known a priori and thus selected as a starting point. The food characteristics can be a food attribute (e.g., color) or a chemical compound (e.g., vitamin C). The analytical methods applied in this approach are specifically optimised for the full quantitative expression of the characteristics (e.g., concentration). However, when considering the complexity of food quality-related reactions, focusing on a (set of) particular chemical reaction(s) or characteristic(s) entails the risk that other important effects can be overlooked (Kebede et al., 2015).

The second approach is the 'untargeted analytical approach' (fingerprinting). This approach considers as many chemical compounds as possible and allows an initial fast screening to detect differences among samples. Since no single analytical methodology is applicable to detect, quantify and identify as much as possible analytes in a given food matrix, no matter what, a specific decision needs to be made on the food fraction to be analysed, for example either a liquid or headspace fraction of the food. Consequently, compared to the targeted approach, the untargeted approach offers a more hypothesis-free technique, however, the quantification is relative to other (control/treated) samples (e.g., relative concentration) and the identification of the unknown compounds is often a challenging task (Grauwet et al., 2014).

The objective of this work is to study the quality changes of pasteurised, shelf-stable mango juice as a function of shelf-life. Because different chemical compositions may lead to different chemical reactions, in our work, besides the control mango juice (mango puree and water), mango juice samples were formulated with addition of different potential precursors for different quality-related chemical reactions (e.g., degradation of ascorbic acid, acid-catalysed degradation of sugars): ascorbic acid, citric acid and sugars. In our previous study, these compounds were linked to changes in targeted quality parameters (e.g., colour, HMF and furfural) of pasteurised orange juice during storage at ambient and elevated temperature (Wibowo et al., 2015b; Wibowo et al., 2015a). Moreover, in the work on orange juice, fingerprinting of the volatile fraction showed potential to investigate different quality-related chemical reactions (Wibowo, Grauwet, Kebede, Hendrickx, & Van Loey, 2015). It is known that volatiles are often involved in process- and storage-induced chemical reactions and can be used as a witness for what is happening in other food fractions (e.g., liquid fraction) (Kebede et al., 2015; Vervoort et al., 2012). Also, the availability of mass spectral libraries of the GC-MS allows preliminary volatile compound identification.

In the current work, to obtain insight in the mango juice quality changes as a function of shelf-life and over the different mango juice compositions, a wide range of quality attributes were evaluated. More particular, we integrated a targeted and untargeted analytical approach, i.e., both targeted (colour, acidity, sugars, oxygen, vitamin C, furfural, HMF and carotenoids) and untargeted (volatile fraction) data were combined into one multivariate data set. In this paper or in **Part I** of this work, multivariate data analysis was used to find shelf-life markers or quality attributes clearly changing as a function of shelf-life. Focus is given to the higher correlation to storage time as indicated by the higher Variable Identification coefficients

(VIDs). From this, a selection of shelf-life markers and their link to different reaction pathways are discussed. In the second paper or in **Part II**, the kinetics of the selected shelf-life markers are studied and modelled in detail.

2 MATERIALS AND METHODS

2.1 Experimental set-up

The experimental set-up used in this work is schematically presented in **Figure 1**. Mango juice was produced as such (control) and at different formulations (section 2.2). Juices were thermally pasteurised and stored at accelerated shelf-life conditions (42 °C) for a period of 8 weeks (section 2.2). ASLT works on the basic assumption that the principles of chemical kinetics can be applied to quantify the effects of extrinsic parameters (e.g., temperature) on the rate of deteriorative chemical reactions (Robertson, 1999). At particular selected time moments, mango juices were sampled. As a function of shelf-life, all mango juices were characterised for a range of targeted quality parameters (section 2.3) as well as for a volatile fingerprint (untargeted, section 2.4). All obtained targeted and untargeted data were combined in one dataset and analysed with multivariate data analysis in order to select those quality characteristics significantly changing as a function of shelf-life (section 2.5).

2.2 Mango juice formulation, processing and storage

Mango juice was prepared in seven different formulations; the first formulation was the control juice, which was prepared by mixing not from concentrate (NFC) mango puree cv. 'Totapuri' (16.7-17.6 °Brix) with water in 1:1 ratio (v/v). The other formulations were prepared by adding 230 mg L^{-1} and 460 mg L^{-1} ascorbic acid (AA_{250} and AA_{500}), 7.8 g L^{-1} citric acid (CA), combination of citric acid and ascorbic acid ($CA+AA_{250}$ and $CA+AA_{500}$) and sugars (S) consisting 9.82 g L^{-1} of fructose, 20.39 g L^{-1} of glucose and 19.52 g L^{-1} of sucrose to the control juice. Conditions for addition of the reaction precursors (e.g., ascorbic acid, citric acid, sugars) were selected based on measured concentration of these precursors in

orange juice (Wibowo et al., 2015a). For every formulation (i.e. control, AA_{250}, AA_{500}, CA, $CA+AA_{250}$, $CA+AA_{500}$ and S), 40 L of mango juice mixture was pasteurised in a tubular heat exchanger at 92 °C during 30 seconds. After processing, 500 mL polyethylene terephthalate (PET) bottles were filled with pasteurised mango juice, including some headspace. Bottles were cooled to ambient temperature by submerging them into a circulating chlorinated water tank. For every formulation, 20 bottles were stored in temperature-controlled incubators (IPP500, Memmert, Schwabach, Germany) at 42 °C for 0, 1, 2, 4, 6 and 8 weeks protected from light. At each sampling time, all samples were transferred into smaller tubes ($\pm$ 30 mL), frozen in liquid nitrogen and stored at –80 °C. Prior to targeted and untargeted analyses, each sample tube was thawed in a circulating water bath at 25 °C.

2.3 Analysis of targeted quality parameters

2.3.1 Colour measurement

Colour of the mango juice samples was evaluated on the basis of CIELAB colour values by using a HunterlabColorQuest 45/0 spectrophotometer (Hunterlab, Reston, Virginia, USA). The instrument (45°/0° geometry, Illuminant D65, 10° observer) was standardised with a black and white ceramic tile (X = 78.66, Y = 83.31, Z = 88.40) before each measurement. 3 mL of sample was poured in a glass cell and covered with a white plate. Colour measurements were carried out in triplicate. The recorded XYZ tristimulus values were then converted to CIE L^*, a^* and b^* colour values. The L^* value (measure of lightness, ranges from 0 (black) to 100 (white)), the a^* value (measure of greenness (-) to redness (+)) and the b^* value (measure of blueness (-) to yellowness (+)) (Chen, Zhu, Zhang, Niu, & Du, 2010). The colour change during storage was expressed as total colour difference (ΔE^*) according to Equation 1, where $\Delta L^* = L^* - L^*_0$, $\Delta a^* = a^* - a^*_0$ and $\Delta b^* = b^* - b^*_0$. The subscript "0" indicates initial colour at week 0. ΔE^* can be classified as not noticeable (0 – 0.5), slightly noticeable (0.5 – 1.5),

noticeable (1.5 – 3.0), well visible (3.0 – 6.0) and great (> 6.0) (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006). Additionally, two other colour parameters, the chroma (C^*_{ab}) or degree of colour saturation or intensity and hue angle (h_{ab}) (measure of red = 0° or 36°, yellow = 90 °, green = 180° and blue = 270°) were calculated by Equation 2 and 3, respectively (CIE, 1978).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h_{ab} = \arctan b^*/a^* \quad (3)$$

2.3.2 Acidity determination

2.3.2.1 pH and titratable acidity

The pH of the mango juice was measured with a pH meter (Meterlab PHM210, Radiometer Analytical, Villeurbanne, France) and the titratable acidity (TA), expressed in percent citric acid, was determined by titration of 10 g of juice with 0.1N NaOH using phenolphthalein as an indicator (AOAC, 1998). Samples were analysed in triplicate.

2.3.2.2 Organic acid profile

Organic acid extraction and chromatographic analysis were based on the method of Vervoort et al. (2011). Ten millilitres of each mango juice sample was transferred into a Nalgene centrifuge tube (50 mL). Clarification was done by adding 500 µL each of Carrez I (15% w/v $K_4[Fe(CN)_6]$ and Carrez II (30% w/v $ZnSO_4$) solutions and homogenisation by a vortex. After resting for 30 min at room temperature, the mixture was centrifuged at a speed of 24000×g for 15 min at 4 °C (J2-HS centrifuge, Beckman, Brea, CA, USA). Prior to injection, samples were filtered through a 0.45 µm syringe filter (Chromafil A-45/25,

Macherey-Nagel, Duren, Germany) and transferred to a dark brown crimp cap vial. Two microlitres of the extract was analysed using reversed phase (RP) high performance liquid chromatography (HPLC) (Agilent 1200 series, Diegem, Belgium), equipped with a Prevail Organic Acid column (250 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, USA) protected with a Prevail C_{18} guard cartridge (7.5 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, USA). An isocratic elution of 25 mM potassium dihydrogen phosphate buffer (pH 2.5) was used at flow rate 1 mL min^{-1} and at 25 °C. The system was equipped with a UV-DAD detector set to a wavelength of 210 nm. All samples were analysed in triplicate. The identification was performed by comparing the UV spectra and retention times of the detected compounds with the values of standard solutions. The quantification was performed by calibration curve based on peak area. For this, standard solutions of a range of organic acids were prepared in milli-Q water. The regression equation for citric acid and malic acid were $y = 67.40x - 5.53$ ($R^2 = 0.99$) and $y = 49.86x - 0.14$ ($R^2 = 0.99$), respectively.

2.3.3 Sugar determination

2.3.3.1 Total sugar content

Total sugar content, expressed as degree Brix, was measured in triplicate using a digital refractometer (RX-7000α, Atago, Tokyo, Japan) at 20 °C.

2.3.3.2 Sugar profile

Samples were extracted analogous to the organic acids (2.3.2.2) method and further analysed using a RP-HPLC system with evaporative light scattering detection (Alltech 3300 ELSD, Grace, Deerfield, IL, USA). Five microlitres of a 10-fold dilution of the extract was separated on a Prevail carbohydrate ES column (analytical column 250 mm x 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, USA) protected with a Prevail C_{18} guard cartridge by isocratic

elution with 75% (v/v) acetonitrile/water at a flow rate of 1 mL min^{-1}. The column was maintained at 30 °C. The drift tube temperature for ELSD was set at 38 °C and nitrogen was used as a nebulizer gas at a flow rate of 1.5 mL min^{-1}. Analyses were carried out in triplicate. Stock solutions of 5 mg mL^{-1} and 0.5 mg mL^{-1} of sugars were prepared in milli-Q water. The identification was performed by comparing the retention times of the detected compounds to those of known standards, while the quantification was performed by calibration curves based on peak area, with regression equation for fructose ($y = 688.38x - 1090.97$, $R^2 = 0.99$), glucose ($y = 451.63x - 1205.81$, $R^2 = 0.99$) and sucrose ($y = 673.55x - 1689.04$, $R^2 = 0.99$).

2.3.4 Determination of the dissolved and headspace oxygen content

The dissolved oxygen and headspace were measured using a non-invasive oxygen analyser system, OxySense® 4000B (OxySense, Las Vegas, NV, USA). The principle of the measurement is based on the fluorescence-based oxygen sensor, known as Oxydot (Mills, 2005). This sensor (O_2xyDots®) was attached inside the PET bottle (in duplicate for each formulation) prior to filling and sealing. First, the system was calibrated by specifying the OxyDot calibration numbers before measurement. Then, the OxyDots were illuminated by a blue LED from the fibre optic reader-pen. Both dissolved and headspace oxygen content were measured five times for each bottle.

2.3.5 Vitamin C: ascorbic acid (AA) and dehydroascorbic acid (DHA) determination

The total vitamin C content of mango juice was determined based on the method described by Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey (2013), with some modifications. Both ascorbic acid (AA) and dehydroascorbic acid (DHA) were extracted by adding 15 mL of extraction buffer (1% w/v meta-phosphoric acid with 0.5% oxalic acid adjusted to pH 2.0) into 5 mL of mango juice. Then, the sample was homogenised using a vortex and deoxygenated by flushing with nitrogen gas for 5 min. The mixture was

centrifuged for 15 min at 24000×g (4 °C). After centrifugation, the supernatant was filtered through a 0.45 μm syringe filter. For the determination of AA content, 1 mL filtrate was mixed with 2 mL of phosphate buffer (20 mM NaH_2PO_4+1 mMNa_2EDTA, pH 3.5) and filtered using a 0.45 μm syringe filter. To determine the DHA content, 1 mL filtrate was mixed with 2 mL of reducing reagent (TCEP 2.5 mM tris (2-carboxyl-ethyl) phosphine in phosphate buffer, pH 3.5) and centrifuged at a speed of 19900×g for 15 min at 4 °C (Microfuge 22R, Beckman Coulter). Then, the supernatant was filtered through a 0.45 μm syringe filter. Finally, the DHA content was calculated by subtracting the total vitamin C content with the AA content.

The chromatographic analysis of vitamin C was performed using a RP-HPLC system (Dionex BiolC, Sunnyvale, CA, USA) equipped with a Prevail C18-column (250×4.6 mm, 5 μm particle size, equilibrated at 25 °C) and with a UV detection system. The mobile phase (1 mM Na_2EDTA and 10 mM CH_3COONH_4) was pumped through the system at a flow rate of 0.8 mL min^{-1}. For all samples, a 25 μL injection volume was used and the detection was performed at 245 nm. The AA content was quantified based on a calibration curve using an external standard solution of AA in extraction buffer. A 10-fold dilution was carried out and from this, different concentrations were prepared. The exact concentration of the standard solutions was determined in accordance with the Beer-Lambert law ($A = \varepsilon \cdot c \cdot l$) by measuring the absorbance of the solutions using a spectrophotometer (245 nm, 25 °C, pH 0.69, ε =10.3 mM^{-1} cm^{-1}). All measurements were done in triplicate.

2.3.6 Furfural and 5-hydroxymethylfurfural (HMF) determination

The analysis of furfural and HMF was based on the method described by Lee et al. (1986), yet

with some modifications. Ten millilitres of juice was mixed with 500 µL Carrez I and 500 µL Carrez II and left to rest for 30 min. After centrifugation (24000×*g* at 4 °C for 15 min), the upper phase was applied on a C18 SPE pre-column (Sep-PAK C-18 Waters), which was previously washed with 2 mL methanol and 5 mL 0.5% acetic acid. The columns were subsequently washed with 2 mL milli-Q water and the investigated compounds were selectively eluted with 4.5 mL ethyl acetate. The eluate was dried with anhydrous sodium sulphate and filtered through a 0.45 µm syringe filter. Prior to HPLC injection (5 µL), the volume was adjusted to 5 mL.

The separation was done on a Zorbax Eclipse XDB C18 column (150 × 4.6 mm, 5 µm particle size, Agilent technologies, Diegem, Belgium), coupled with a Prevail C18 guard column. The temperature of the column was maintained at 25 °C. The column was eluted with a mixture of acetonitrile/water (10/90 (v/v)) at a flow rate of 1 mL min^{-1}. The detection was performed at 277 nm (furfural) and 285 nm (HMF). For quantification purposes, a standard solution containing furfural and HMF (1.5 mg mL^{-1}) was prepared in 10% (v/v) methanol. The calibration curve was built by plotting peak area against known concentration. The regression equation was $y = 8883.71x - 0.42$ ($R^2 = 0.99$) for furfural and $y = 8401.21x + 0.02$ ($R^2 = 0.99$) for HMF. The identification of furfural and HMF was performed by comparing the spectra and retention times of the detected compounds with the values of commercial standard solutions.

2.3.7 Carotenoid content determination

The determination of the carotenoid content was based on a procedure described by Lemmens, Tchuenche, Van Loey & Hendrickx (2013), with some modifications. All extraction steps were carried out under subdued light to avoid photo-isomerisation and photo-

degradation. Five grams of mango juice was mixed with 2 g $CaCl_2$ and 30 mL extraction solvent (hexane/ethanol/acetone, 50/25/25 (v/v/v), containing 0.1% butylated hydroxytoluene (BHT) (w/v)) for 20 min at 4 °C using a stirrer at 500 rpm. Next, 10 mL of milli-Q water was added to the mixture and stirred for 15 min at 4 °C. Afterwards, the mixture was transferred to a separatory funnel and the aqueous phase was removed. The extract was washed two times with 10 mL aqueous NaCl (10%) to remove any trace of acetone and followed by a saponification step using 15 mL of 10% (w/v) ethanolic KOH (containing 0.1% (w/v) BHT). After 1 h, the remaining traces of alkali were removed by washing the extract three times with NaCl (10%). The extract, containing the carotenoids, was collected and filtered using a 0.20 µm syringe filter (Chromafil PET-20/25, Macherey-Nagel, Düren, Germany).

Analyses were carried out by injecting 10 µL of the filtrate into a RP-HPLC system equipped with a C30-reversed phase column (150 × 4.6 mm, 3 µm particle size, YMC Europe, Dinslaken, Germany) equilibrated at 25 °C. Gradient elution of methanol (A), methyl-t-butylether (B) and milli-Q water (C) was applied for a period of 24 min at a flow rate of 1 mL min^{-1}. The initial condition was 81 % A, 15 % B and 4 % C and the end condition was 41 % A, 55 % B and 4 % C. The diode array detector (DAD) was set at 450 nm, the wavelength of maximal carotenoid absorbance. The identification of carotenoids was performed by comparing the retention times and spectra of the detected compounds with the ones of commercial standards. The quantification was performed based on the calibration curve of all-*trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene and 15-*cis*-β-carotene standard (CaroteNature, Lupsingen, Switzerland) solutions with concentrations ranging from 2 – 20 µg mL^{-1}. The total carotenoid content was calculated as the sum of the individually detected carotenoid contents.

2.4 Untargeted headspace SPME-GC-MS fingerprinting

The headspace volatile fraction of mango juice was analysed according to the method of Liu et al. (2014a). Amber glass vials (10 mL, VWR International, Radnor, PA, USA) were filled with 5 g mango juice and 1.8 g NaCl. Vials were tightly closed using screw-caps with silicone septum seal (GRACE, Columbia, MD, USA), homogenised and placed in the cooling tray of the CombiPAL autosampler (CTC analytics, Zwingen, Switzerland) at 10 °C. After an equilibrium step for 20 min at 30 °C (500 rpm), volatiles at the vial headspace were extracted using SPME fibres coated with 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (StableFlex, Supelco, Bellefonte, PA, USA) at 40 °C for 10 min. Previously, these fibres were conditioned according to the manufacturer's guidelines. Analysis was carried out on a gas chromatography (GC) system (7890N Agilent technologies, Diegem, Belgium) coupled to a mass selective detector (MSD) (5977N, Agilent Technologies, Diegem, Belgium). Volatiles were desorbed at 230 °C for 2 minutes from the fibre into the injector port of the GC–MS, which operated in split (1/5) mode. Using helium as a carrier gas at at constant flow of 1.2 mL min^{-1}, the volatiles were separated on a HP-5MS capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies J&W, Santa Clara, CA, USA). The initial oven temperature was set at 40 °C. After 2 min, the oven temperature was raised to 160 °C at a rate of 4 °C min^{-1} and then ramped to 300 °C at a rate of 50 °C $min^{-1.}$ The temperature was maintained at 300 °C for 2 min and cooled back to 40 °C. The mass spectra were obtained by electron ionization (EI) mode with electron energy of 70 eV at a scanning range of 35–400 m/z and a scanning speed of 3.8 scans per seconds. MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively.

A new fibre was used for each formulation. During analysis, the samples were randomised as a function of storage time per formulation. Possible fibre degradation was monitored by

analysis of a reference sample (pasteurised mango juice at week 0), every 14 injections. The peak area of the reference samples was investigated as a function of time to monitor the performance of the SPME fibre and the GC-MS. In the present work, deviation of the peak area of the reference samples was less than 5% during the analysis, which shows limited fibre degradation and a good performance of the instrument (results not shown).

2.5 Data analysis

As described in **Figure 1**, data from targeted and untargeted analyses were combined into one data set. In this paper, all data were analysed by a multivariate analytical approach to determine which quality parameters (targeted) and headspace volatile components (untargeted) significantly changed during shelf-life at 42 °C. Changes in targeted and untargeted characteristics were investigated in two ways: (*i*) investigating the common trends over all mango juice formulations as a function of shelf-life and (*ii*) investigating the specific quality evolution per formulation. The kinetic modelling of the changes of the selected shelf-life markers obtained from this study will be further discussed in the follow up manuscript (**Part II**: Kinetic modelling of the shelf-life markers).

2.5.1 Data pre-processing

All headspace SPME-GC-MS total ion chromatograms were analysed using an Automated Mass Spectral Deconvolution and Identification System (AMDIS) (Version 2.66, 2008, National Institute of Standards and Technology, Gaithersburg, MD, USA) to extract "pure" component spectra from complex chromatograms. AMDIS was also used to build a retention index calibration file. The retention index along with the mass spectral data was further used for data compound identification. Next, the deconvoluted mass spectra were analysed with a Mass Profiler Professional (MPP) (Version 12.0, 2012, Agilent Technologies, Diegem, Belgium) software for filtering and peak alignment. Per formulation, the resulting

spreadspheet containing peak areas was combined with targeted quality parameter data obtained as described in section 2.3 (colour, acidity, sugars, oxygen, vitamin C, furfural, HMF and carotenoids). This integrated data table was used as an input for the multivariate data analysis.

2.5.2 Multivariate data analysis

A multivariate data analysis was applied (Solo Version 6.5, 2011, Eigenvector Research, Wenatchee, WA) to investigate which targeted quality parameters and volatile components (untargeted) are changing the most during storage. Firstly, all data were mean-centered and the variables were weighed by their standard deviation to give them equal variance, followed by principal component analysis (PCA) to evaluate each data set (per formulation) and to detect potential outliers. Subsequently, the changes during storage were investigated by partial least squares (PLS) regression. The acidity (pH, titratable acidity and organic acid profile), sugars (total sugar content and sugar profile), oxygen (headspace and dissolved), vitamin C (ascorbic and dehydroascorbic acid), furfural, HMF, total carotenoids and detected volatile compounds were considered as *X*-variables and storage time as continuous *Y*-variable. The multivariate model consisting of the lowest number of latent variables (LVs) resulting in class separation was selected. In other words, latent variables were added to the model until they at least contributed more than 2% of the Y-variance of the selected model. The performance of the PLS model was evaluated based on the root mean square error of calibration (RMSEC) and root mean square error of cross-validation (RMSECV) including leave-one-sample-out cross-validation. In order to visualise the detected changes during storage, biplots of scores and correlation loadings described by the first two latent variables were constructed for each comparison performed using OriginPro 8 (Origin Lab Corporation, Northampton, MA). To quantitatively rank volatiles' and quality parameters' importance for change as a function of storage time, Variable Identification (VID) coefficients were subsequently calculated. In this

work, to evaluate common trends over all mango juice formulations as a function of shelf-life, variables with the higher absolute VID values indicate they have higher correlation with storage time and are defined as shelf-life markers. In this part, all formulations were referred as one class (section 0). To investigate specific quality changes per formulation, variables with an absolute VID value higher than 0.90 were found to significantly change during storage (section 3.2) and were further zoomed into (section 3.3). Tentative identification of the volatiles was carried out by comparing the deconvoluted mass spectrum with the reference mass spectra from the NIST spectral library (NIST08, version 2.0, National Institute of Standards and Technology, Gaithersburg, MD, USA) and WILEY mass spectra data (WILEY2010 version 9, Hoboken, New York, USA). For identification purpose, a threshold match of 90 % was taken into account. In addition, for confirmation purpose, a visual inspection of the spectral matching between the detected compound and the match from the library as well as comparison of the retention index were performed.

3 RESULTS AND DISCUSSION

In our work, seven different mango juice formulations were prepared by adding potential precursors for different quality-related chemical reactions (e.g., degradation of ascorbic acid, acid-catalysed degradation of sugars): ascorbic acid (AA), citric acid (CA) and sugars (S), as shown from the result of our previous shelf-life study in orange juice (Wibowo et al., 2015b; Wibowo et al., 2015a). All mango juice samples were stored at accelerated storage temperature conditions of 42 °C for 8 weeks. The control (mango pure and water), ascorbic acid-added (AA250 and AA500), citric acid-added (CA, CA+AA250 and CA+AA500) and sugar-added (S) samples were characterised for a range of targeted quality parameters as well as for a volatile fingerprint (untargeted). All obtained targeted and untargeted data were combined in one dataset and analysed with multivariate data analysis. Results are discussed in three parts: (i) investigation of the common shelf-life quality changes over all formulations (section 3.1), (ii) investigation of the specific shelf-life quality changes per mango juice formulation. In the last section, (iii) the interpretation of the selected shelf-life markers are made by discussing them with the literature data (section 3.3). Changes in the selected volatile compounds are discussed as relevant chemical groups (terpene hydrocarbons, terpene alcohols, terpene oxides, terpene phenols, sulphur compounds, acids, ketones and esters).

3.1 Investigation of the common shelf-life quality changes over all mango juice formulations

The common trends over all mango juice samples during 8 weeks of storage at 42 °C was investigated using a multivariate PLS model, in which all quality attributes considered as *X*-variables and storage time as continuous *Y*-variable. In this section, the type of formulation was neglected or, in other words, all formulations were classifed into one class. A biplot with

two first latent variables (LVs) explaining 80.6% of the *Y*-variance, is shown in **Figure 2**. A clear effect of shelf-life was observed in the direction of the horizontal axis, as indicated by the first LV (67.6% *Y*-variance explained). Storage time is represented by the colour scale from grey to black and different formulations are indicated by differently shaped symbols.

On the biplot, the importance of the targeted quality parameters and volatile fraction (untargeted) for quality change during storage can be qualitatively represented by their location and their distance from the centre. A quality parameter or volatile component can be considered to change prominent when it is located between the two ellipses (inner and outer ellipses indicate correlation coefficients of 70% and 100%). Parameters and volatiles projected far away from the center and in the same direction as the *Y*-vector are positively correlated with storage time, indicating increase in concentration or value as a function of storage time. Conversely, the ones that are far away in the opposite direction are negatively correlated and their concentrations or values decrease during storage. In **Figure 2**, the trend over all formulations can be seen, in which more quality parameters and volatiles (small open circles) are projected to the end of storage than to the start of storage.

Since a biplot is not a straightforward tool to indicate the most important quality parameters and volatile components for shelf-life changes, Variable Identification (VID) coefficients were calculated. The VID coefficients are the correlation coefficients between each original *X*-variable and predicted *Y*-variable. Each volatile and quality parameter was assigned with a value between -1 and +1, in which a positive VID coefficient represents an increase in concentration or value after storage and a negative coefficient denotes a decrease. The higher the absolute VID value, the higher the correlation is. As shown in **Figure 2**, there were 4 targeted quality parameters with absolute value higher than 0.80, lightness (L^*), yellowness

(b^*), hue (h_{ab}) and the total colour difference (ΔE^*). It was observed that during storage at 42 °C, the L^*, b^* and h_{ab} values decreased while ΔE^* increased. The decrease in L^* and b^* values for all formulations indicates a decrease in yellowness and brightness of mango juice samples during storage. As ΔE^* quantifies the magnitude of colour difference between the stored sample and the initial sample (week 0), an increase in ΔE^* values means more visually differences in stored juice than at the start of storage.

Additionally, two volatile compounds belonging to the group of terpene hydrocarbons were selected by the VID procedure and seemed to decrease during shelf-life, δ-cadinene (VID = -0.82) and α-pinene (VID = -0.83). α-pinene, a monoterpene compound, is responsible for the pine like-odour and δ-cadinene, a sesquiterpene compound, has a mild and dry wood-like odour (Lalel, Singh, & Tan, 2003; Munafo, Didzbalis, Schnell, Schieberle, & Steinhaus, 2014). Both compounds are among other volatile compounds which can be found during the ripening of mango fruit (Lalel et al., 2003).

In **Figure 2**, in addition to the horizontal direction, there is also a variation in the vertical direction. Overlaping of samples was observed at the beginning of storage, followed by a clear separation towards the end of storage. This separation occuring in the direction of the vertical axis could indicate the effect of formulation. As indicated by differently shaped symbols for different mango juice formulations, it was observed that the control, the ascorbic acid-enriched (AA_{250} and AA_{500}) and the sugar-enriched samples (S) are grouped together as well as all citric acid-enriched samples (CA, CA+AA_{250} and CA+AA_{500}). The average pH and titratability acid values, respectively, were 4.02 and 0.26% of citric acid for the control, the ascorbic acid-enriched and the sugar-enriched samples and 3.10 and 0.83% of citric acid for the citric acid-enriched samples. As this difference in acidity might evoke different chemical

reactions during storage, changes in quality attributes per mango formulation were further zoomed into in the next section.

3.2 Investigation of the specific shelf-life quality changes per mango juice formulation

To investigate the specific quality evolution during shelf-life per mango juice formulation, PLS models were built and biplots were constructed for each formulation separately (control, AA_{250}, AA_{500}, CA, CA+AA_{250}, CA+AA_{500} and S) (**Figure 3**). The variance in the *Y*-variables explained by the first two LVs was 98.3%, 99.1%, 99.4%, 99.5%, 99.4%, 99.6% and 99.4% for formulation control, AA_{250}, AA_{500}, CA, CA+AA_{250}, CA+AA_{500} and S, respectively. Similarly to the observation in **Figure 2**, a noticeable evolution of quality during storage of mango juices was observed in the biplots. Moreover, the relation between each component and storage time can be described by a biplot. It appears that more quality parameters and volatiles are projected to the end of shelf-life than to the beginning of shelf-life, or in other words more quality parameters and volatiles increased in value or in concentration as a function of storage compared to the ones being decreased.

A more quantitative measure based on calculation of VID coefficients was applied to select quality parameters and volatiles clearly changing during shelf-life. Per formulation, variables with an absolute VID value higher than 0.90 were selected and further zoomed into. The identification of the volatile compounds was based on the available mass spectral libraries. The selected shelf-life markers, which are defined as 'components clearly changing during storage', are listed in **Table 1** and marked as open bold circles in **Figure 3**. 15 out of 21 targeted quality parameters were found to significantly change during storage of 8 weeks at 42 °C: colour coordinates (L^* a^*, b^*, ΔE^*, C^*_{ab} and h_{ab}), sugars (°Brix, fructose, glucose and sucrose), oxygen (dissolved and headspace oxygen), ascorbic acid, furfural and HMF. Other parameters, such as citric acid, malic acid, titratable acidity, dehydroascorbic acid and total

carotenoids were not changing significantly during storage. An increase in concentration could be observed for fructose, glucose, furfural and HMF as well as an increase in °Brix, ΔE^* and a^* values, while sucrose, ascorbic acid, dissolved oxygen, headspace oxygen, L^*, b^*, C^*_{ab} and h_{ab} were decreasing as a function of storage.

Besides the aforementioned targeted quality parameters, there were 35 detected volatile compounds clearly changing during storage. The selected volatile compounds can be classified as terpene hydrocarbons (e.g., α-terpinolene), terpene alcohols (e.g., ocimenol and myrcenol), terpene oxides (e.g., linalool oxide), terpene phenols (*p*-cymene-2-ol or carvacrol), sulphur compounds (dimethyl sulphide), furfural, acids (butanoic acid), ketones (e.g., 3-penten-2-one) and esters (e.g., ethyl acetate). In general, more volatile compounds were found with an important increasing trend in concentration or value as a function of storage compared to the ones being characterised by a decrease. As marked in bold italic in **Table 1**, some volatiles such as terpene alcohols (ocimenol and myrcenol) and oxides (linalool oxide) were found to increase in concentration (as indicated by positive VID values) irrespective of mango juice formulations, which could imply that these compounds are generally being formed during shelf-life at 42 °C. On the contrary, it was observed that terpene hydrocarbons (e.g., α-pinene) and ester compounds (e.g., ethyl butanoate) being degraded at each formulation (note: ester compounds were detected at VID lower than -0.80 for C, AA_{250}, AA_{500} and S samples). It should be taken into account that variables with absolute VID values lower than 0.90 do not mean that the variables did not change during storage. Those with higher absolute VID values imply a higher correlation with storage time.

Referring to **Table 1**, remarkable differences can be observed between samples with the addition of citric acid (CA, CA+AA_{250} and CA+AA_{500}) and without citric acid (control,

AA_{250}, AA_{500} and S). As indicated by the positive VID coefficients, the addition of citric acid seems to influence the formation of terpene oxides such as cineole or 1,8-cineole; isocineole or 1,4-cineole; ocimene quintoxide or tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)-furan; linaloyl oxide or 2,2,6-trimethyl-6-vinyltetrahydropyran); and caryophyllan-2,6-β-oxide. Moreover, only in citric acid-enriched formulations, β-terpineol and γ-terpineol (terpene alcohols) and α-myrcene (terpene hydrocarbons) were markers with an increasing concentration during storage. The most possible explanation for the difference in volatile fraction during storage between samples with and without citric acid is probably that the lower pH favours particular reaction pathways.

When evaluating the influence of ascorbic acid on the evolution of the volatile fraction and quality parameters, it was observed that furfural was significantly changing for ascorbic acid-enriched samples (AA_{250}, AA_{500}, $CA+AA_{250}$ and $CA+AA_{500}$). In these samples, furfural data from both HPLC (targeted) and/or HS-SPME-GC-MS (untargeted) were selected by the VID procedure (note: as can be seen in **Table 1**, two furfural data appear in the $CA+AA_{250}$ and $CA+AA_{500}$ samples). Furthermore, the ascorbic acid content decreased more significantly in the AA samples compared to control, CA and S samples.

3.3 Interpretation of the selected shelf-life markers

To gain insight into the different mechanisms responsible for the detected quality changes in stored mango juice samples, in the following section, it was aimed to discuss the selected quality parameters from the integrated targeted and untargeted multivariate approach in the context of different possible reaction pathways described in literature.

3.3.1 Changes in targeted quality parameters

3.3.1.1 Changes in colour

It was observed that all colour parameters L^*, a^*, b^*, ΔE^*, C^*_{ab} and h_{ab} were clearly changing during storage at 42 °C (**Table 1**). L^* (lightness), b^* (blueness to yellowness), C^*_{ab} (colour intenstity) and h_{ab} (hue) values were decreasing, while a^* (greenness to redness) and ΔE^* (the total colour difference) values were increasing. Similar with the visual observation, the decrease in L^* and b^* indicates that the yellowness and brightness of all mango juice samples decreased. An increase in ΔE^* values indicates increasing colour differences during storage as compared to the start of storage. The effect of storage time on colour was also investigated by Vásquez-Caicedo Schilling, & Carle (2007) for canned mango puree and by Liu, Wang, Li, Bi, & Liao (2014b) for PET-bottled mango nectar. These authors observed colour degradation or browning during storage in mango based-products.

As colour is the result of the presence of several compounds absorbing or reflecting light of a certain wavelength, it is difficult to state which compound is reponsible for the observed changes. A direct correlation between colour and pigment compounds such as carotenoids and anthocyanins was reported (Sant'Anna, Gurak, Ferreira, & Tessaro, 2013). Moreover, changes in colour can be used as an indicator for different chemical and biochemical reactions (van Boekel, 2009). Some proposed mechanisms for colour changes during storage are discussed in literature, such as the formation of coloured compounds due to polymerisation of intermediate degradation products from ascorbic acid or sugars (Zerdin, Rooney, & Vermuë, 2003; Robertson & Samaniego, 1986). Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici (2000) reported that non-sugar molecules with carbonyl groups (e.g., ascorbic acid, phenols and lipid oxidation products) can react with amino groups complicating the pathway of the browning reactions. In the study of Vásquez-Caicedo et al. (2007), the oxidation of

polyphenols promoted by the presence of oxygen and the residual peroxidase activity was responsible for the colour deterioration of canned mango puree during storage.

3.3.1.2 Changes in sugars

Three types of sugars were monitored in this study: sucrose, fructose and glucose. During storage at 42 °C, sucrose concentration decreased while fructose and glucose concentration increased in each mango juice formulation. This observation was in accordance with the study of Lee & Nagy (1988), who observed more than 50 % of sucrose loss in grapefruit juice after six weeks of storage at 30 °C. Changes of sugars have been associated with the acid-catalysed sucrose inversion and the Maillard reaction, which lead to HMF formation (Liu, Li, Wang, Bi, & Liao, 2014; Kennedy, Rivera, Lloyd, Warner, & Jumel, 1990; Lee & Nagy, 1988). The type of sugar is one of the factors that influence the HMF formation, in which fructose was reported to be five times more reactive than glucose (Lee & Nagy, 1990a). In the present work, HMF was found to significantly change during storage in all formulations (section 3.3.1.5). The total sugar content of mango juice can be expressed as °Brix. An increase in °Brix was clearly detected for the citric acid-enriched (CA and CA+AA_{250}) and sugar samples (S) (**Table 1**). A possible explanation could be that other soluble degradation products whose formation is catalysed under acid condition, could be formed during storage. In contrast to the observed increase in °Brix, Falade, Babalola, Akinyemi, & Ogunlade (2004) reported a slightly decrease in °Brix of sweetened mango (cv. 'Julie' and 'Ogbomoso') juice during 12 weeks of storage at 25 °C.

3.3.1.3 Changes in oxygen

A decrease in both headspace and dissolved oxygen during storage for all mango juice samples was observed (**Table 1**). This clearly indicates that oxygen was consumed during oxidation reaction(s). One of the compounds that was affected by oxygen is ascorbic acid

(section 3.3.1.4). Aerobic degradation of ascorbic acid occurs in the presence of oxygen with further formation of decomposition products such as furfural (section 3.3.1.5). Other oxidative chemical reactions that could be related with oxygen consumption are for example oxidative degradation of carotenoids, terpenes and lipids (Rodriguez-Amaya, 2001; Turek & Stintzing, 2013; Ullrich & Grosch, 1987). These reactions influence not only changes in the sensorial properties but affect also the nutritional value of the juice.

3.3.1.4 Changes in ascorbic acid

Ascorbic acid (AA) clearly decreased upon storage, particularly in mango juice samples with the addition of ascorbic acid (AA_{250}, AA_{500}, $CA+AA_{250}$ and $CA+AA_{500}$). A decrease in ascorbic acid concentration during storage was observed in mango nectar (Liu, Wang, Li, Bi, & Liao, 2014b) and in fresh-cut mango (Gonzalez-Aguilar et al., 2008). Several researchers associated a decrease in ascorbic acid concentration during storage with aerobic and anaerobic degradation reaction pathways of ascorbic acid (Robertson et al., 1986; Zerdin et al., 2003). Hence, the ascorbic acid degradation is correlated with the oxygen permeability of the packaging material (Berlinet, Brat, Brillouet, & Ducruet, 2006). In our study, all mango juice formulations were filled in PET bottles with headspace, therefore, oxygen can diffuse from the headspace into the liquid fraction as well as from the environment through the packaging. If the packaging has high oxygen permeability, such as in monolayer PET bottles, the aerobic pathway of AA degradation can predominate. It was observed that both headspace and dissolved oxygen were decreasing during storage in all mango juice formulations, which could indicate the oxygen consumption by ascorbic acid degradation reaction and other oxidative reactions (section 3.3.1.3). Loss of ascorbic acid is not only important in terms of the nutritional value but also in relation to flavour change and browning (Lee et al., 1988; Nagy & Randall, 1973).

3.3.1.5 Changes in furfural and 5-hydroxymethylfurfural (HMF)

In the current work, both furfural and HMF were formed during 8 weeks of storage at 42 °C. Similarly, the increased levels of furfural in mango juice after 16 weeks at different storage temperatures was observed by Ibrahim, Salem, & Abdallah (1975). In our work, furfural was detected to increase significantly in ascorbic acid-enriched samples (AA_{250}, AA_{500}, $CA+AA_{250}$ and $CA+AA_{500}$) (**Table 1**). According to Robertson & Samaniego (1986), furfural can be formed from ascorbic acid and sugars. In literature, it was hypothesised that the aerobic degradation of ascorbic acid results in furfural through a series of reactions: hydration of dehydroascorbic acid to ketogulonic and followed by decarboxylation and dehydration leads to the formation of furfural (Kanner, Harel, Fishbein, & Shalom, 1981). In the current work, the concentration of ascorbic acid, the precursor for furfural formation, decreased particularly at AA_{250}, AA_{500}, $CA+AA_{250}$ and $CA+AA_{500}$ samples (section 3.3.1.4). The increase in HMF concentration was observed in all mango juice formulations. Changes in HMF during storage could be linked to the Maillard reaction and acid-catalysed dehydration and cyclisation of hexoses (Arena, Fallico, & Maccarone, 2001).

Furfural and HMF are important quality indicators of a wide-range of food productsduring processing and storage. Both compounds have been associated to browning of fruit juices (Robertson et al., 1986). According to Zerdin et al. (2003), furfural is capable of reacting with amino acids and undergoes polymerisation to form brown pigments. Also, a strong correlation between HMF and browning formation during storage of orange juice was reported (Robertson et al., 1986). From a regulation point of view, a maximum limit of 5 to 10 mg L^{-1} of HMF in fruit juices was established by the International Federation of Fruit Juice Processors (IFFJP) (Gaspar & Lucena, 2009).

3.3.2 Changes in volatile fraction (untargeted)

3.3.2.1 Changes in terpene hydrocarbons

In the present study, terpene hydrocarbons represented the major group of volatile compounds in all mango juice samples. A representative GC-MS total ion chromatogram of the headspace fraction of control juice sample at the beginning of storage is presented in **Figure 4**, the three most abundant peaks could be identified as β-myrcene, followed by β-ocimene and α-pinene. The presence of β-myrcene as the most abundant component in mango juice samples was in agreement with the study of Pandit et al. (2009), who reported that β-myrcene contributed to 51% of the total amount of volatiles (204 μg g^{-1}) in 'Totapuri' mango. However, differences in the main volatile compounds among different mango cultivars can be found, for example δ-3-carene in 'Haden' (Pino, Mesa, Muñoz, Martí, & Marbot, 2005), α-terpinolene in 'Kensington Pride' (Macleod, Macleod, & Snyder, 1988), α-pinene in 'Vallenato' (Quijano, Salamanca, & Pino, 2007) and (*Z*)-ocimene in 'Alphonso' (Idstein & Schreier, 1985).

During 8 weeks of storage at 42 °C, among the monoterpene hydrocarbons (C_{10}), α-pinene, β-*cis*-ocimene, α-terpinolene, α-myrcene, *p*-cymene, α-terpinene and camphene was detected to significantly change during storage. The sesquiterpene hydrocarbons (C_{15}) group represented by β-caryophyllene, δ-cadinene and δ-selinene did also change. An increase in terpene hydrocarbons was observed for β-*cis*-ocimene, α-terpinolene, α-myrcene, *p*-cymene, α-terpinene, δ-selinene and camphene, whereas a decrease was observed for α-pinene, β-caryophyllene and δ-cadinene (**Table 1**). Some compounds were affected depending on the formulation, for example, α-myrcene significantly increased in the citric acid-enriched samples (CA, CA+AA_{250} and CA+AA_{500}) and camphene in the ascorbic acid-enriched samples (AA_{250}, AA_{500}), as well as in the control and sugar enriched-samples (S) with VID higher than +0.80. While, α-pinene decreased in all formulations (note: as well as in control

and CA samples with VID < -0.80) (section 0). Changes in these volatiles could be attributed to the acid-catalysed hydrolysis and rearrangement reactions of other terpene hydrocarbons. In lime oil, the hydrolysis and rearrangement of bicyclic hydrocarbons (e.g., α- and β-pinene, sabinene and α-thujene) produce not only terpene alcohols such as α-terpineol, terpinen-4-ol, α-fenchol, borneol and isoborneol, but also terpene hydrocarbons terpinolene, camphene, γ-terpinene and α-terpinene (Chamblee & Clark, 1997). The accumulation of *p*-cymene can be due to the acid-catalysed cyclisation of citral (Schieberle & Grosch, 1988) or oxidative dehydrogenation of single or double unsaturated monocyclic terpenes (Turek et al., 2013). This compound was reported among others as an off-odour compound in citrus juices (Perez-Cacho et al., 2008), with oxidised and woody odour (Cheong, Liu, Zhou, Curran, & Yu, 2012).

3.3.2.2 Changes in terpene alcohols

Terpene alcohols such as α-terpineol, α-fenchol, borneol, isoborneol, ocimenol, myrcenol were observed to increase during storage, as indicated by positive VID values (**Table 1**). Compounds such as ocimenol and myrcenol were selected in all formulations, while α-terpineol was selected in the samples without citric acid (control, AA_{250}, AA_{500} and S). An increase in terpene alcohols during storage was in line with the observation of El-Nemr, Ismail, & Askar (1988), who reported an increase in terpene alcohols (terpinene-4-ol, α-terpineol, sellin-11-en-4-ol) in mango juice after 4 months of storage at 20-25 °C. In literature, different pathways are described to be linked to the formation of terpene alcohols, for example the formation of *p*-cymene-8-ol was reported by Schieberle & Grosch (1988) through cyclisation and oxidation reactions in a low-pH condition, which decreased the citral concentration. In addition, the acid-catalysed degradation of α- and β-pinene was shown to result in the formation of off-odour terpene alcohol compounds such as borneol and α-fenchol (Lebossé, Ducruet, & Feigenbaum, 1997). Other researchers investigated the formation of α-

terpineol from limonene and linalool (Haleva-Toledo, Naim, Zehavi, & Rouseff, 1999; Perez-Cacho et al., 2008). Moreover, the increase of α-terpineol can occur via hydrolysis of esters (neryl and geranyl acetate), followed by isomerisation and cyclisation of its hydrolysis products, nerol and geraniol (Chamblee et al., 1997). α-terpineol can be transformed into 1,8-terpine and later to 1,8-cineole (Farina, Boido, Carrau, Versini, & Dellacassa, 2005) (section 3.3.2.3)

3.3.2.3 Changes in terpene oxides

Upon storage at 42 °C, an increase in terpene oxides was observed. Linalool oxide changed significantly in all mango juice formulations. On the other hand, several oxides were clearly formed in the samples with the addition of citric acid (CA, CA+AA_{250} and CA+AA_{500}), namely 1,8-cineole, 1,4-cineole, ocimene quintoxide, linaloyl oxide and caryophyllan-2,6-β-oxide. This could indicate that the formation of these compounds was enhanced under acidic condition. In the study of Choi & Sawamura (2002), an increase in terpene oxides such as limonene oxide, linalool furanoxide and caryophyllene oxide during storage of citrus oil was observed at 30 °C after one week. The high acidity of a fruit juice, such as in lemon juice, seems to favour the degradation reaction of linalool, which formed several compounds, including 1,8-cineole (Haleva-Toledo et al., 1999). Moreover, limonene and α-terpineol could act as a precursor of 1,8-cineole (Farina et al., 2005). They reported that at pH 3.2 and 45 °C, limonene can be hydrated to α-terpineol and followed by cyclisation of *trans*-1,8-terpine to form 1,8-cineole. Another suggested pathway is that the epoxidation of the double bonds of limonene and α-terpineol resulting in a complex mixture of α-terpinolene, 1,4-cineole and 1-terpineol. In relation to temperature effects, terpene oxides are commonly detected in heated juice and not in fresh juice (Williams, Strauss, & Wilson, 1980) and their concentrations are more evident for juices stored at higher storage temperatures than at lower temperatures (Choi & Sawamura, 2002).

3.3.2.4 Changes in terpene phenols

One volatile phenol compound, *p*-cymene-2-ol or carvacrol, was detected to increase during storage, particularly in control and sugar-enriched samples. A previous study has shown that volatile phenols such as thymol, 4-vinylguaiacol, eugenol and carvacrol were detected in a trace amount in various mango cultivars (Pino et al., 2005). However, the mechanisms of the formation of volatile phenols during storage in mango juice are not clearly known. In processed orange juice, 2-methoxy-4-vinylphenol (also known as 4-vinylguaiacol) was reported to increase during storage (Averbeck & Schieberle, 2011; Tatum, Nagy, & Berry, 1975). It has been hypothesised that 4-vinylguaiacol is formed through a decarboxylation reaction of free ferulic acid, in which this compound should be released first from its bound forms (e.g., glycosides, esters and amides). Moreover, thermal processing, acidity and subsequent high storage temperature appear to favour the release process (Lee & Nagy, 1990b). As several phenolic compounds such as gallic acid, gallotanins, *p*-coumaric acid and ferulic acid were identified in mango fruit (Kim, Lounds-Singleton, & Talcott, 2009), these compounds may potentially serve as precursor for the phenol volatiles. Averbeck & Schieberle (2011) reported that 4-vinylguaiacol exhibited a clove-like odour and the increase of this compound together with changes in other key aroma compounds may be responsible for the overall aroma changes in orange juice during storage.

3.3.2.5 Changes in sulphur compounds

It was observed that a sulphur-containing compound, namely dimethyl sulphide, increased during storage in all formulations, with a VID value higher than +0.80. The presence of sulphur compounds in various fresh ripe mango cultivars was previously reported, such as dimethyl sulphide (Macleod et al., 1988), diallyl disulfide and diallyl trisulfide (Pino et al., 2005), dimethyl trisulfide, (*E*)-2-butene-1-thiol and 3-methyl-2-butene-1-thiol (Munafo et al.,

2014). The formation of sulphur volatiles during storage can be linked to the oxidation of methanethiol. In literature, the precursors of the sulphur compounds in canned orange juice are possibly the sulphur-containing amino acids (e.g., methionine), which via Strecker degradation releases methional and methanethiol (Perez-Cacho, Mahattanatawee, Smoot, & Rouseff, 2007). The sulphur volatiles are known as off-odour compounds with sulphurous and cabbage-like odour (Munafo et al., 2014; Perez-Cacho et al., 2008).

3.3.2.6 Changes in acids

The concentration of volatile acids did increase during 8 weeks of storage at 42 °C. An increase in carboxylic acid (butanoic acid) was observed in the citric acid-enriched samples ($CA+AA_{250}$ and $CA+AA_{500}$) (note: butanoic acid was also detected at VID = +0.81 in CA samples). This was in agreement with the study of Averbeck et al. (2011), who observed an increase in acetic acid, butanoic acid and hexanoic acid in orange juice after four weeks of storage at 37 °C. The formation of acids could be attributed to hydrolysis of esters into carboxylic acid and alcohol (Ayhan, Zhang, & Min, 2002) (section 3.3.2.8). Butanoic acid is described having a rancid odour by Averbeck et al. (2011).

3.3.2.7 Changes in ketones

Two ketone compounds were selected as shelf-life markers in pasteurised mango juice (**Table 1**), 3-penten-2-one in all formulations (VID values higher than +0.80), and 6-methyl-3,5-heptadien-2-one in sugar-enriched samples. The formation of ketone compounds during processing and storage was investigated by different researchers. Ketones such as 6-methyl-3,5-heptadien-2-one and 6-methyl-5-hepten-2-one were detected in higher amount in thermally-treated tomato paste than in fresh tomato (Buttery, Teranishi, Ling, & Turnbaugh, 1990). In paprika oleoresin, the formation of 6-methyl-3,5-heptadien-2-one from β-carotene was due to an addition mechanism at position 13, 14 followed by heterolytic fragmentation.

Furthermore, lycopene, γ-, δ- and ζ-carotene were also reported as the precursors for this compound (Rios, Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2008). Carvone, known as the off-flavour ketone, was formed through an oxidation of limonene and its concentration increased during thermal treatment and oxidative storage (Perez-Cacho et al., 2008). Moreover, breakdown of lipids contributes to a wide range of secondary and tertiary products, including aldehydes and ketones. As an example, an autooxidation product of linoleic acid and methyl linoleate, 1-octen-3-one, was identified as one of the most intense odour-active compounds, imparting metallic and mushroom-like notes (Perez-Cacho et al., 2008; Ullrich et al., 1987). Besides their relation to processing and storage, lipids also play an important role in determining the aroma characteristic of mango during ripening, particularly with respect to the fatty acids composition (Bandyopadhyay & Gholap, 1973). They reported that 'Totapuri' mango has a higher ratio of palmitic to palmitoleic acid than 'Alphonso' mango, of which when the ratio is below one, the fruit has a strong aroma and if above one, the fruit has a mild aroma.

3.3.2.8 Changes in esters

Three ester compounds were decreasing during storage (butyl butanoate, ethyl acetate and ethyl butanoate) (**Table 1**). A similar observation was reported by Averbeck & Schieberle (2011) in stored reconstituted orange juice. More than 40% reduction in ethyl butanoate was found after storage for 16 weeks at 37 °C. Another study conducted by Golaszewski, Sims, O'Keffe, Braddock, & Littell (1998) also reported a decrease in esters (isopropyl butyrate and butyl acetate) of stored strawberry juice. The decrease in esters during storage could be linked to the hydrolysis of esters into alcohol and acids, as referred by Ayhan, Zhang, & Min (2002). Esters such as ethyl butanoate, ethyl 3-methylbutanoate and ethyl 2-methylpropanoate are known for their contribution to the fruity note in different cultivars of mango fruits (Munafo et al., 2014; Pino & Mesa, 2006).

4 CONCLUSION

Quality changes of pasteurised mango juice during 8 weeks of storage at 42 °C were investigated by integration of a targeted and untargeted multivariate approach. An evaluation was made to investigate, on the one hand, common trends over all formulations and on the other hand, to investigate specific quality changes per formulation. As graphically represented by a biplot, colour coordinates (e.g., L^*, b^*, ΔE^* and h_{ab}) were correlated the highest with storage time irrespective of the formulation. Additionally, two terpene hydrocarbon compounds (δ-cadinene and α-pinene) were selected. Zooming in to changes per formulation individually: sugars (°Brix, fructose, glucose and sucrose), oxygen (dissolved and headspace oxygen), ascorbic acid, furfural and HMF showed the most important changes during storage. Furthermore, it was observed that 35 volatile compounds, which classified as terpene hydrocarbons, terpene alcohols, terpene oxides, terpene phenols, sulphur compounds, furfural, acids, ketones and esters, were significantly changing during storage. Some of these compounds were significantly changing and showed a clear distinct behavior in a particular formulation. This implies that their degradation or formation pathways are influenced by the mango juice composition. Relating to the possible reaction pathways responsible for the detected differences, under more acidic conditions (CA, CA+AA_{250}, CA+AA_{500}), oxides were formed significantly, which could indicate that their formation was favoured by acid. Moreover, in samples with addition of ascorbic acid (AA_{250}, AA_{500}, CA+AA_{250}, CA+AA_{500}), furfural and ascorbic acid showed a stronger correlation with storage time. As discussed in the literature, among all volatile compounds, only furfural is related with browning, while most of the selected volatile markers contribute to the odour quality rather than the visual quality. Concerning the consumer point of view, changes in the volatiles compounds, particularly the increased levels of off-odour compounds (e.g., dimethyl sulphide and α-terpineol) could determine the acceptance of the product, therefore, monitoring these compounds would be

important. In order to evaluate changes of the selected volatile compounds and quality parameters, the kinetic behaviour of these selected shelf-life markers obtained in the present study will be discussed in the following manuscript (**Part II**: Kinetic modelling of the shelf-life markers).

Acknowledgement

This research was financially supported by the Seventh Framework Programme (FP7) of the European Union under the Marie Curie Initial Training Network 'HST FoodTrain' (Grant agreement 264470). Tara Grauwet is a researcher funded by the Research Foundation Flanders (FWO) as well as the KU Leuven.

References

Aguiló-Aguayo, I., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. *LWT - Food Science and Technology, 42,* 813-818.

AOAC (1998). *Official methods of analysis of AOAC International (16th ed.)*. Arlington, VA, USA: AOAC International.

Arena, E., Fallico, B., & Maccarone, E. (2001). Thermal damage in blood orange juice: kinetics of 5-hydroxymethyl-2-furancarboxaldehyde formation. *International Journal of Food Science & Technology, 36,* 145-151.

Averbeck, M. & Schieberle, P. (2011). Influence of different storage conditions on changes in the key aroma compounds of orange juice reconstituted from concentrate. *European Food Research and Technology, 232,* 129-142.

Ayhan, Z., Zhang, Q. H., & Min, D. B. (2002). Effects of pulsed electric field processing and storage on the quality and stability of single-strength orange juice. *Journal of Food Protection, 65,* 1623-1627.

Bandyopadhyay, C. & Gholap, A. S. (1973). Relationship of aroma and flavour characteristics of mango (Mangifera indica L.) to fatty acid composition. *Journal of the Science of Food and Agriculture, 24,* 1497-1503.

Berlinet, C., Brat, P., Brillouet, J. M., & Ducruet, V. (2006). Ascorbic acid, aroma compounds and browning of orange juices related to PET packaging materials and pH. *Journal of the Science of Food and Agriculture, 86,* 2206-2212.

Burdurlu, H. S. & Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry, 80,* 91-97.

Buttery, R. G., Teranishi, R., Ling, L. C., & Turnbaugh, J. G. (1990). Quantitative and sensory studies on tomato paste volatiles. *Journal of Agricultural and Food Chemistry, 38,* 336-340.

Chamblee, T. S. & Clark, B. C. (1997). Analysis and chemistry of distilled lime oil (Citrus aurantifolia Swingle). *Journal of Essential Oil Research, 9,* 267-274.

Chen, Z., Zhu, C., Zhang, Y., Niu, D., & Du, J. (2010). Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (Lactuca sativa L.). *Postharvest Biology and Technology, 58,* 232-238.

Cheong, M. W., Liu, S. Q., Zhou, W., Curran, P., & Yu, B. (2012). Chemical composition and sensory profile of pomelo (Citrus grandis (L.) Osbeck) juice. *Food Chemistry, 135,* 2505-2513.

Choi, H. S. & Sawamura, M. (2002). Effects of storage conditions on the composition of citrus tamurana Hort. ex Tanaka (Hyuganatsu) essential oil. *Bioscience, Biotechnology, and Biochemistry, 66,* 439-443.

CIE (1978). Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms. *CIE Publication No.15 (E-1.3.1) 1971, Supplement 2*.

Cserhalmi, Zs., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science and Emerging Technologies, 7,* 49-54.

El-Nemr, S. E., Ismail, I. A., & Askar, A. (1988). Aroma changes in mango juice during processing and storage. *Food Chemistry, 30,* 269-275.

Falade, K. O., Babalola, S. O., Akinyemi, S. O. S., & Ogunlade, A. A. (2004). Degradation of quality attributes of sweetened Julie and Ogbomoso mango juices during storage. *Eur Food Res Technol, 218,* 456-459.

FAOSTAT (2012). *Production quantity of mangoes, mangosteens and guavas [online]. http://faostat3.fao.org. [Last accessed on 31/01/2015]*.

Farina, L., Boido, E., Carrau, F., Versini, G., & Dellacassa, E. (2005). Terpene compounds as possible precursors of 1,8-Cineole in red grapes and wines. *Journal of Agricultural and Food Chemistry, 53,* 1633-1636.

Gaspar, E. M. S. M. & Lucena, A. F. F. (2009). Improved HPLC methodology for food control - furfurals and patulin as markers of quality. *Food Chemistry, 114,* 1576-1582.

Golaszewski, R., Sims, C. A., O'Keffe, S. F., Braddock, R. J., & Littell, R. C. (1998). Sensory attributes and volatile components of stored strawberry juice. *Journal of Food Science, 63,* 734-738.

Gonzalez-Aguilar, G. A., Celis, J., Sotelo-Mundo, R. R., de la Rosa, L. A., Rodrigo-Garcia, J., & varez-Parrilla, E. (2008). Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5°C. *International Journal of Food Science & Technology, 43,* 91-101.

Grauwet, T., Vervoort, L., Colle, I., Van Loey, A., & Hendrickx, M. (2014). From fingerprinting to kinetics in evaluating food quality changes. *Trends in Biotechnology, 32,* 125-131.

Haleva-Toledo, E., Naim, M., Zehavi, U., & Rouseff, R. L. (1999). Formation of α-terpineol in citrus juices, model and buffer solutions. *Journal of Food Science, 64,* 838-841.

Ibrahim, S. S., Salem, F. M. A., & Abdallah, M. A. (1975). Egyptian mango juice. Experiment on storage experience. *Food / Nahrung, 19,* 207-211.

Idstein, H. & Schreier, P. (1985). Volatile constituents of alphonso mango (Mangifera Indica). *Phytochemistry, 24,* 2313-2316.

Kanner, J., Harel, S., Fishbein, Y., & Shalom, P. (1981). Furfural accumulation in stored orange juice concentrates. *Journal of Agricultural and Food Chemistry, 29,* 948-949.

Kebede, B. T., Grauwet, T., Magpusao, J., Palmers, S., Michiels, C., Hendrickx, M. et al. (2015). An integrated fingerprinting and kinetic approach to accelerated shelf-life testing of chemical changes in thermally treated carrot puree. *Food Chemistry, 179,* 94-102.

Kennedy, J. F., Rivera, Z. S., Lloyd, L. L., Warner, F. P., & Jumel, K. (1990). Studies on non-enzymic browning in orange juice using a model system based on freshly squeezed orange juice. *Journal of the Science of Food and Agriculture, 52,* 85-95.

Kim, Y., Lounds-Singleton, A. J., & Talcott, S. T. (2009). Antioxidant phytochemical and quality changes associated with hot water immersion treatment of mangoes (Mangifera indica L.). *Food Chemistry, 115,* 989-993.

Lalel, H. J. D., Singh, Z., & Tan, S. C. (2003). Aroma volatiles production during fruit ripening of 'Kensington Pride' mango. *Postharvest Biology and Technology, 27,* 323-336.

Lebossé, R., Ducruet, V., & Feigenbaum, A. (1997). Interactions between reactive aroma compounds from model citrus juice with polypropylene packaging film. *Journal of Agricultural and Food Chemistry, 45,* 2836-2842.

Lee, H. S. & Nagy, S. (1990a). Relative reactivities of sugars in the formation of 5-Hydroxymethylfurfural in sugar-catalyst model systems. *Journal of Food Processing and Preservation, 14,* 171-178.

Lee, H. S. & Nagy, S. (1988). Quality changes and nonenzymic browning intermediates in grapefruit juice during storage. *Journal of Food Science, 53,* 168-172.

Lee, H. S. & Nagy, S. (1990b). Formation of 4-vinyl fuaiacol in adversely stored orange juice as measured by an improved HPLC method. *Journal of Food Science, 55,* 162-163.

Lee, H. S., Rouseff, R. L., & Nagy, S. (1986). HPLC determination of furfural and 5-hydroxymethylfurfural in citrus juices. *Journal of Food Science, 51,* 1075-1076.

Lemmens, L., Tchuenche, E. S., Van Loey, A. M., & Hendrickx, M. E. (2013). Beta-carotene isomerisation in mango puree as influenced by thermal processing and high-pressure homogenisation. *Eur Food Res Technol, 236,* 155-163.

Liu, F., Grauwet, T., Kebede, B. T., Van Loey, A., Liao, X., & Hendrickx, M. (2014a). Comparing the efects of high hydrostatic pressure and thermal processing on blanched and unblanched mango (Mangifera indica L.) nectar: using headspace fingerprinting as an untargeted approach. *Food Bioprocess Technol, 7,* 3000-3011.

Liu, F., Li, R., Wang, Y., Bi, X., & Liao, X. (2014). Effects of high hydrostatic pressure and high-temperature short-time on mango nectars: Changes in microorganisms, acid invertase, 5-hydroxymethylfurfural, sugars, viscosity, and cloud. *Innovative Food Science & Emerging Technologies, 22,* 22-30.

Liu, F., Wang, Y., Li, R., Bi, X., & Liao, X. (2014b). Effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and color of mango nectars. *Innovative Food Science & Emerging Technologies, 21,* 35-43.

Macleod, A. J., Macleod, G., & Snyder, C. H. (1988). Volatile aroma constituents of mango (cv Kensington). *Phytochemistry, 27,* 2189-2193.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2000). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science & Technology, 11,* 340-346.

Mills, A. (2005). Oxygen indicators and intelligent inks for packaging food. *Chemical Society Reviews, 34,* 1003-1011.

Munafo, J. P., Didzbalis, J., Schnell, R. J., Schieberle, P., & Steinhaus, M. (2014). Characterization of the major aroma-active compounds in mango (Mangifera indica L.) cultivars haden, white alfonso, praya sowoy, royal special, and malindi by application of a comparative aroma extract dilution analysis. *Journal of Agricultural and Food Chemistry, 62,* 4544-4551.

Nagy, S. & Randall, V. (1973). Use of furfural content as an index of storage temperature abuse in commercially processed orange juice. *Journal of Agricultural and Food Chemistry, 21,* 272-275.

Pandit, S. S., Chidley, H. G., Kulkarni, R. S., Pujari, K. H., Giri, A. P., & Gupta, V. S. (2009). Cultivar relationships in mango based on fruit volatile profiles. *Food Chemistry, 114,* 363-372.

Perez-Cacho, P. R., Mahattanatawee, K., Smoot, J. M., & Rouseff, R. (2007). Identification of sulfur volatiles in canned orange juices lacking orange flavor. *Journal of Agricultural and Food Chemistry, 55,* 5761-5767.

Perez-Cacho, P. R. & Rouseff, R. (2008). Processing and storage effects on orange juice aroma: A review. *Journal of Agricultural and Food Chemistry, 56,* 9785-9796.

Pino, J. A. & Mesa, J. (2006). Contribution of volatile compounds to mango (Mangifera indica L.) aroma. *Flavour and Fragrance Journal, 21,* 207-213.

Pino, J. A., Mesa, J., Muñoz, Y., Martí, M. P., & Marbot, R. (2005). Volatile components from mango (Mangifera indica L.) cultivars. *Journal of Agricultural and Food Chemistry, 53,* 2213-2223.

Polydera, A. C., Stoforos, N. G., & Taoukis, P. S. (2005). Quality degradation kinetics of pasteurised and high pressure processed fresh Navel orange juice: Nutritional parameters and shelf life. *Innovative Food Science and Emerging Technologies, 6,* 1-9.

Quijano, C. E., Salamanca, G., & Pino, J. A. (2007). Aroma volatile constituents of Colombian varieties of mango (Mangifera indica L.). *Flavour and Fragrance Journal, 22,* 401-406.

Rawson, A., Patras, A., Tiwari, B. K., Noci, F., Koutchma, T., & Brunton, N. (2011). Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. *Food Research International, 44,* 1875-1887.

Rios, J. J., Fernández-García, E., Mínguez-Mosquera, M. I., & Pérez-Gálvez, A. (2008). Description of volatile compounds generated by the degradation of carotenoids in paprika, tomato and marigold oleoresins. *Food Chemistry, 106,* 1145-1153.

Robertson, G. L. (1999). Shelf Life of Packaged Foods, Its Measurement and Prediction. In *Developing New Food Products for a Changing Marketplace* (CRC Press.

Robertson, G. L. & Samaniego, C. M. L. (1986). Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *Journal of Food Science, 51,* 184-187.

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in food*. Washington: ILSI Press.

Sant'Anna, V., Gurak, P. D., Ferreira, M. L. D., & Tessaro, I. C. (2013). Tracking bioactive compounds with colour changes in foods-A review. *Dyes and Pigments, 98,* 601-608.

Schieberle, P. & Grosch, W. (1988). Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *Journal of Agricultural and Food Chemistry, 36,* 797-800.

Siddiq, M., Akhtar, S., & Siddiq, R. (2012). Mango processing, products and nutrition. In M.Siddiq (Ed.), *Tropical and subtropical fruits:Postharvest physiology, Processing and Packaging* (pp. 277-298). John Willey and Sons, Inc.

Silva, F. V. M. & Gibbs, P. (2004). Target selection in designing pasteurization processes for shelf-stable high-acid fruit products. *Critical Reviews in Food Science and Nutrition, 44,* 353-360.

Singh, Z., Singh, R. K., Sane, V. A., & Nath, P. (2013). Mango - Postharvest Biology and Biotechnology. *Critical Reviews in Plant Sciences, 32,* 217-236.

Tatum, J. H., Nagy, S. T. E. V., & Berry, R. E. (1975). Degradation products formed in canned single-strength orange juice during storage. *Journal of Food Science, 40,* 707-709.

Turek, C. & Stintzing, F. C. (2013). Stability of essential oils: A review. *Comprehensive Reviews in Food Science and Food Safety, 12,* 40-53.

Ullrich, F. & Grosch, W. (1987). Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z Lebensm Unters Forch, 184,* 277-282.

van Boekel, M. A. J. S. (2009). *Kinetic modeling of reactions in foods*. CRC Press-Boca Raton.

Vásquez-Caicedo, A. L., Schilling, S., & Carle, R. (2007). Impact of packaging and storage conditions on colour and β-carotene retention of pasteurised mango purée. *European Food Research and Technology, 224,* 581-590.

Verbeyst, L., Bogaerts, R., Van der Plancken, I., Hendrickx, M., & Van Loey, A. (2013). Modelling of vitamin C degradation during thermal and high-pressure treatments of red fruit. *Food Bioprocess Technol, 6,* 1015-1023.

Vervoort, L., Grauwet, T., Kebede, B. T., Van der Plancken, I., Timmermans, R. A. H., Hendrickx, M. et al. (2012). Headspace fingerprinting as an untargeted approach to compare novel and traditional processing technologies: a case-study on orange juice pasteurisation. *Food Chemistry, 134,* 2303-2312.

Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M. et al. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part II: Impact on specific chemical and biochemical quality parameters. *Innovative Food Science and Emerging Technologies, 12,* 466-477.

Wibowo, S., Grauwet, T., Kebede, B. T., Hendrickx, M., & Van Loey, A. (2015). Study of chemical changes in pasteurised orange juice during shelf-life: A fingerprinting-kinetics evaluation of the volatile fraction. *Food Research International, 75,* 295-304.

Wibowo, S., Grauwet, T., Santiago, J. S., Tomic, J., Vervoort, L., Hendrickx, M. et al. (2015a). Quality changes of pasteurised orange juice during storage: A kinetic study of specific parameters and their relation to colour instability. *Food Chemistry, 187,* 140-151.

Wibowo, S., Vervoort, L., Tomic, J., Santiago, J. S., Lemmens, L., Panozzo, A. et al. (2015b). Colour and carotenoid changes of pasteurised orange juice during storage. *Food Chemistry, 171,* 330-340.

Williams, P. J., Strauss, C. R., & Wilson, B. (1980). Hydroxylated linalool derivatives as precursors of volatile monoterpenes of muscat grapes. *Journal of Agricultural and Food Chemistry, 28,* 766-771.

Zerdin, K., Rooney, M. L., & Vermuë, J. (2003). The vitamin C content of orange juice packed in an oxygen scavenger material. *Food Chemistry, 82,* 387-395.

Table 1. Mango juice targeted quality parameters and volatiles (untargeted) significantly changing as a function of storage time, per formulation, selected based on the VID procedure, listed in decreasing order of VID coefficient. Positive VID coefficients indicate an increase in values while negative coefficients denote a decrease. Samples were stored at 42 °C for 8 weeks: Control samples (mango puree and water), control with addition of ascorbic acid up to 250 mg L^{-1} (AA_{250}), control with addition of ascorbic acid up to 500 mg L^{-1} (AA_{500}), control with addition of citric acid (CA), control with addition of citric acid and ascorbic acid up to 250 mg L^{-1} ($CA+AA_{250}$), control with addition of citric acid and ascorbic acid up to 500 mg L^{-1} ($CA+AA_{500}$) and control with addition of sugars (S). The retention index (RI) and chemical group are listed. Quality parameters (targeted) and volatiles (untargeted) that appeared in all formulations are indicated in bold italic.

Control				AA_{250}				AA_{500}				CA			
VID	**Identity**	**RI**	**Chemical group**	**VID**	**Identity**	**RI**	**Chemical group**	**VID**	**Identity**	**RI**	**Chemical group**	**VID**	**Identity**	**RI**	**Chemical group**
0.99	α-fenchol	981	terpene (alcohol)	0.99	glucose			0.99	glucose			0.99	***ocimenol***	1020	terpene (alcohol)
0.99	borneol	1031	terpene (alcohol)	0.99	***ΔE****			0.99	***ocimenol***	1020	terpene (alcohol)	0.99	1,8-cineole	901	terpene (oxide)
0.98	***ΔE****			0.99	α-fenchol	981	terpene (alcohol)	0.99	borneol	1031	terpene (alcohol)	0.99	isoborneol	1022	terpene (alcohol)
0.98	***myrcenol***	989	terpene (alcohol)	0.99	borneol	1031	terpene (alcohol)	0.98	fructose			0.98	***linalool oxide***	942	terpene (oxide)
0.98	glucose			0.98	***ocimenol***	1020	terpene (alcohol)	0.98	α-terpinolene	957	terpene (hydrocarbon)	0.98	α-myrcene	852	terpene (hydrocarbon)
0.98	α-terpineol	1054	terpene (alcohol)	0.98	***myrcenol***	989	terpene (alcohol)	0.98	furfural	669	aldehydes	0.98	***ΔE****		
0.97	***ocimenol***	1020	terpene (alcohol)	0.98	***5-hydroxymethyl furfural***			0.98	***myrcenol***	989	terpene (alcohol)	0.97	***myrcenol***	989	terpene (alcohol)
0.96	***linalool oxide***	942	terpene (oxide)	0.98	α-terpinolene	957	terpene (hydrocarbon)	0.98	***ΔE****			0.97	1,4-cineole	886	terpene (oxide)
0.96	*p*-cymen-2-ol	1124	terpene (phenol)	0.97	***linalool oxide***	942	terpene (oxide)	0.97	linalool	970	terpene (alcohol)	0.96	***5-hydroxymethyl furfural***		
0.96	isoborneol	1022	terpene (alcohol)	0.97	α-terpineol	1054	terpene (alcohol)	0.97	α-fenchol	981	terpene (alcohol)	0.95	*p*-cymene	895	terpene (hydrocarbon)
0.95	α-terpinolene	957	terpene (hydrocarbon)	0.97	3-penten-2-one	572	ketone	0.96	3-penten-2-one	572	ketone	0.94	*p*-cymen-8-ol	1050	terpene (alcohol)
0.95	***5-hydroxymethyl furfural***			0.96	fructose			0.96	***5-hydroxymethyl furfural***			0.94	dimethyl sulfide	487	sulphur compounds
0.95	3-penten-2-one	572	ketone	0.96	furfural			0.95	α-terpineol	1054	terpene (alcohol)	0.93	3-penten-2-one	572	ketone
0.94	linalool	970	terpene (alcohol)	0.95	isoborneol	1022	terpene (alcohol)	0.94	***linalool oxide***	942	terpene (oxide)	0.93	linaloyl oxide	844	terpene (oxide)
0.93	fructose			0.95	linalool	970	terpene (alcohol)	0.93	isoborneol	1022	terpene (alcohol)	0.93	ocimene quintoxide	919	terpene (oxide)
0.93	*p*-cymen-8-ol	1050	terpene (alcohol)	0.92	δ-selinene	1357	terpene (hydrocarbon)	0.92	camphene	820	terpene (hydrocarbon)	0.93	γ-terpineol	1061	terpene (alcohol)
				0.92	camphene	820	terpene (hydrocarbon)	0.92	α-terpinene	887	terpene (hydrocarbon)	0.93	β-*cis*-ocimene	921	terpene (hydrocarbon)
												0.92	°Brix		
												0.92	β-terpineol	1011	terpene (alcohol)
												0.90	glucose		
–0.93	headspace oxygen			–0.91	headspace oxygen			–0.91	δ-cadinene	1290	terpene (hydrocarbon)	–0.90	β-caryophyllene	1262	terpene (hydrocarbon)
–0.96	dissolved oxygen			–0.93	α-pinene	807	terpene (hydrocarbon)	–0.95	***h_{ab}***			–0.92	***sucrose***		
–0.97	***sucrose***			–0.93	δ-cadinene	1290	terpene (hydrocarbon)	–0.96	α-pinene	807	terpene (hydrocarbon)	–0.93	dissolved oxygen		
–0.98	***h_{ab}***			–0.96	ascorbic acid			–0.96	ascorbic acid			–0.94	***h_{ab}***		
–0.98	*b**			–0.97	***sucrose***			–0.98	***sucrose***			–0.96	butyl butanoate	870	ester
–0.99	*C^*_{ab}*			–0.98	*C^*_{ab}*			–0.98	*b**			–0.97	ethyl acetate	506	ester
–0.99	***L****			–0.98	***h_{ab}***			–0.98	*C^*_{ab}*			–0.97	ethyl butanoate	633	ester
				–0.98	*b**			–0.98	***L****			–0.97	headspace oxygen		
				–0.99	***L****							–0.97	***L****		
												–0.99	***b****		
												–0.99	*C^*_{ab}*		

CA+AA$_{250}$				CA+AA$_{500}$				S			
VID	**Identity**	**RI**	**Chemical group**	**VID**	**Identity**	**RI**	**Chemical group**	**VID**	**Identity**	**RI**	**Chemical group**
0.99	***ocimenol***	1020	terpene (alcohol)	0.99	furfural			0.99	α-fenchol	981	terpene (alcohol)
0.99	isoborneol	1022	terpene (alcohol)	0.99	furfural	669	aldehydes	0.98	borneol	1031	terpene (alcohol)
0.99	furfural			0.98	1,8-cineole	901	terpene (oxide)	0.98	α-terpinolene	957	terpene (hydrocarbon)
0.99	1,8-cineole	901	terpene (oxide)	0.98	*p*-cymen-8-ol	1050	terpene (alcohol)	0.98	α-terpineol	1055	terpene (alcohol)
0.98	***linalool oxide***	942	terpene (oxide)	0.98	isoborneol	1022	terpene (alcohol)	0.97	**Δ*E****		
0.98	*p*-cymen-8-ol	1050	terpene (alcohol)	0.98	***myrcenol***	989	terpene (alcohol)	0.96	6-methyl-3,5-heptadien-2-one	974	ketone
0.98	**Δ*E****			0.98	***linalool oxide***	942	terpene (oxide)	0.96	***5-hydroxymethyl furfural***		
0.98	furfural	669	aldehydes	0.97	***ocimenol***	1020	terpene (alcohol)	0.96	***myrcenol***	989	terpene (alcohol)
0.97	***myrcenol***	990	terpene (alcohol)	0.97	γ-terpineol	1061	terpene (alcohol)	0.95	fructose		
0.96	***5-hydroxymethyl furfural***			0.96	***5-hydroxymethyl furfural***			0.95	3-penten-2-one	572	ketone
0.96	γ-terpineol	1061	terpene (alcohol)	0.96	**Δ*E****			0.95	glucose		
0.95	α-myrcene	852	terpene (hydrocarbon)	0.96	α-myrcene	852	terpene (hydrocarbon)	0.95	***linalool oxide***	942	terpene (oxide)
0.94	butanoic acid	618	carboxylic acid	0.95	1,4-cineole	886	terpene (oxide)	0.94	*p*-cymen-2-ol	1124	terpene (phenol)
0.94	*a**			0.94	ocimene quintoxide	919	terpene (oxide)	0.93	β-*cis*-ocimene	920	terpene (hydrocarbon)
0.94	α-terpinene	887	terpene (hydrocarbon)	0.93	*a**			0.93	linalool	970	terpene (alcohol)
0.93	linaloyl oxide	844	terpene (oxide)	0.93	α-terpinene	887	terpene (hydrocarbon)	0.92	***ocimenol***	1020	terpene (alcohol)
0.92	ocimene quintoxide	919	terpene (oxide)	0.93	butanoic acid	618	carboxylic acid	0.91	°Brix		
0.90	°Brix			0.93	linaloyl oxide	844	terpene (oxide)				
				0.93	caryophyllan-2,6-β-oxide	1257	terpene (oxide)				
–0.91	α-pinene	807	terpene (hydrocarbon)	–0.90	α-pinene	807	terpene (hydrocarbon)	–0.91	dissolved oxygen		
–0.93	***sucrose***			–0.94	***sucrose***			–0.91	α-pinene	807	terpene (hydrocarbon)
–0.94	ethyl acetate	506	ester	–0.96	ethyl acetate	506	ester	–0.93	h_{ab}		
–0.96	ascorbic acid			–0.97	ascorbic acid			–0.93	headspace oxygen		
–0.96	ethyl butanoate	633	ester	–0.98	h_{ab}			–0.94	δ-cadinene	1290	terpene (hydrocarbon)
–0.96	C^*_{ab}			–0.99	***L****			–0.96	C^*_{ab}		
–0.97	*b**							–0.96	*b**		
–0.99	h_{ab}							-0.97	***L****		
–0.99	***L****							–0.98	***sucrose***		

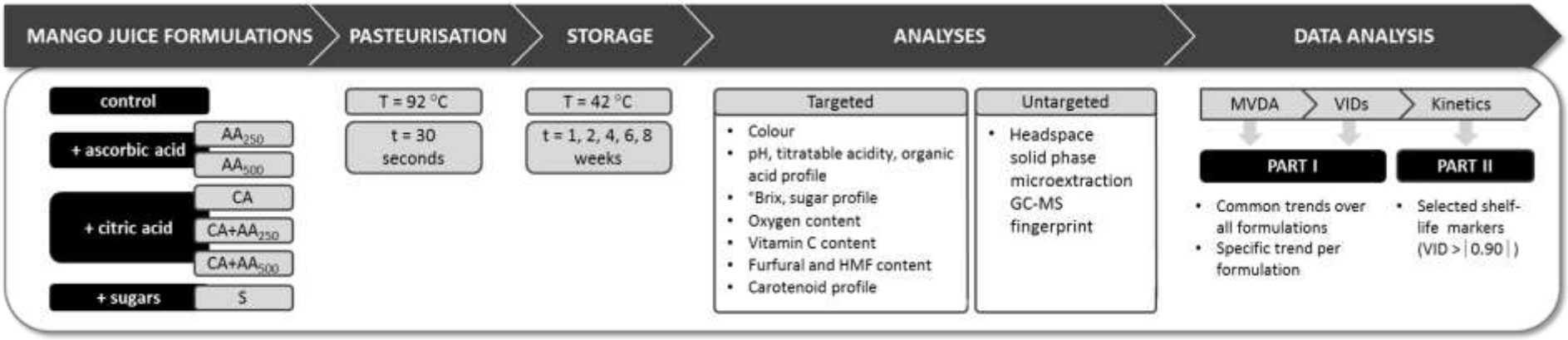


Figure 1. Schematic overview of the mango juice experimental set-up, consisting of formulations, pasteurisation and storage conditions, list of quality parameters analysed and consecutive data analysis approach. In part I, all obtained targeted and untargeted data were analysed by multivariate data analysis (MVDA). By the use of variable identification coefficients (VIDs), targeted quality parameters and volatiles (untargeted) clearly changing during shelf-life could be selected. In part II, the shelf-life changes of selected markers are further zoomed into, in specific kinetic studies.

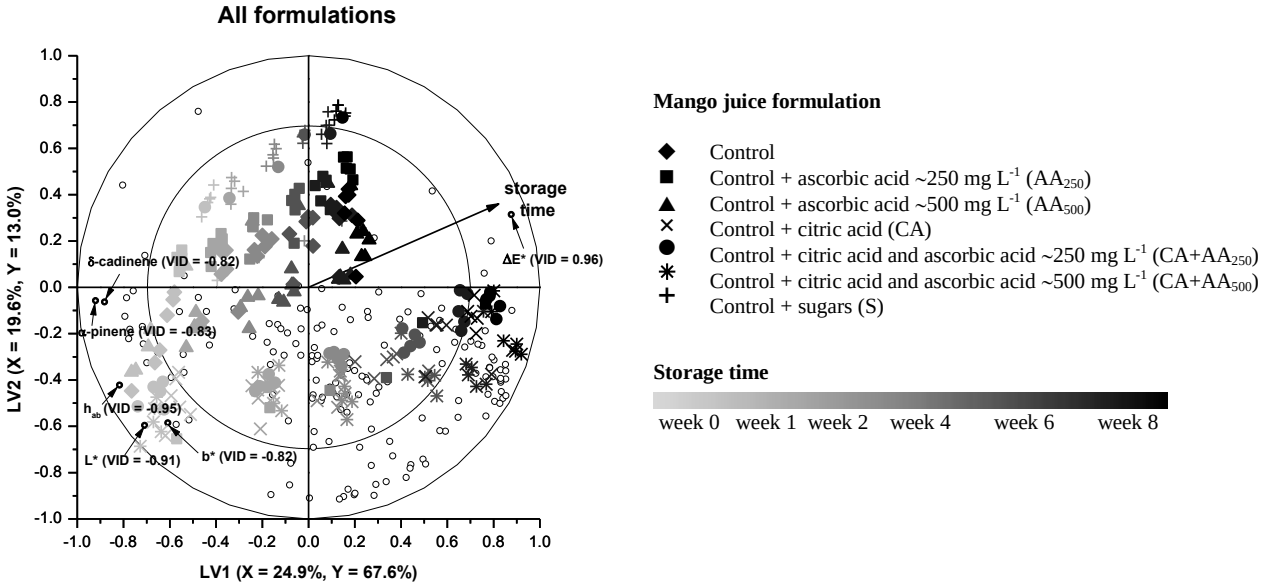
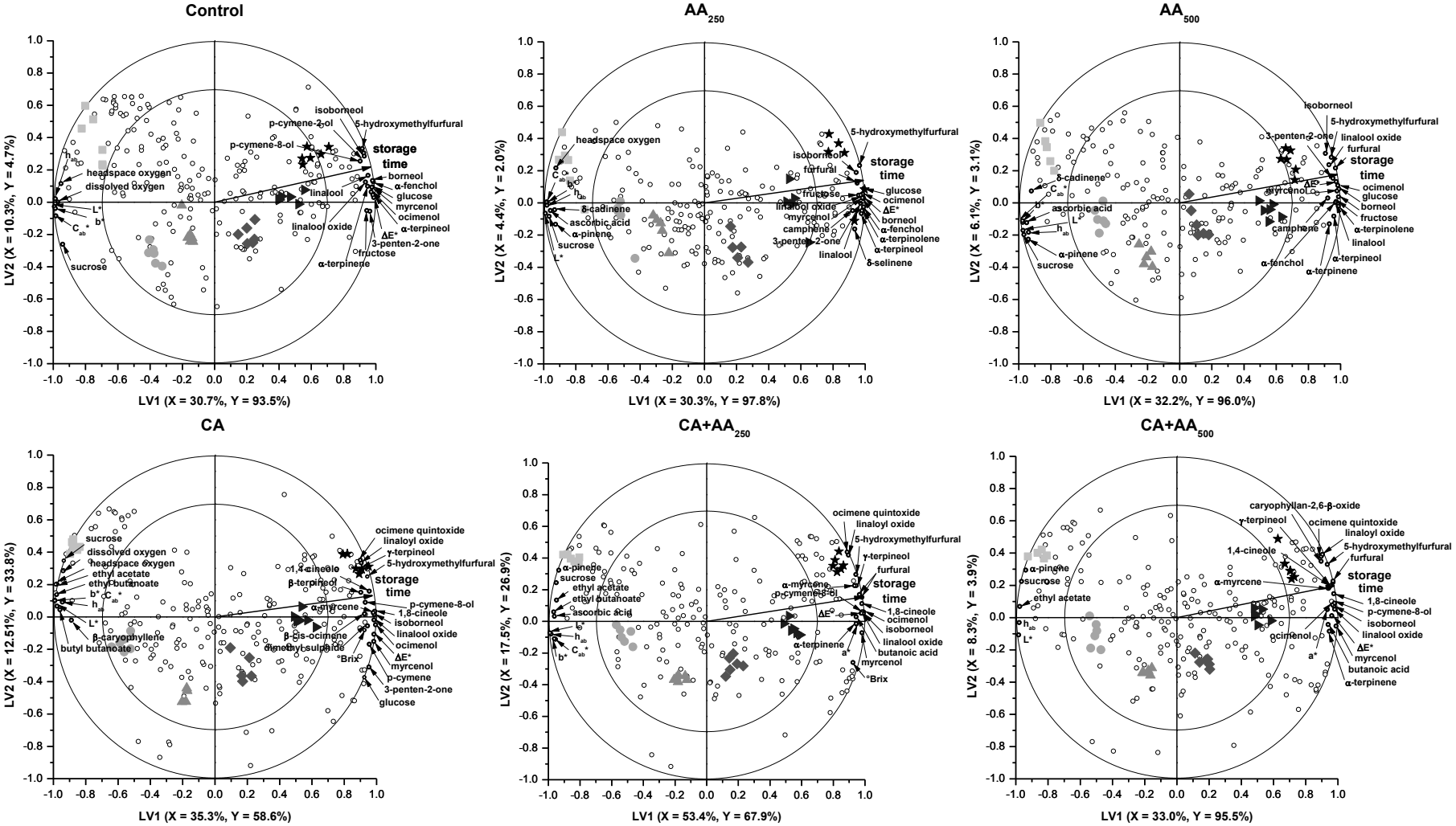
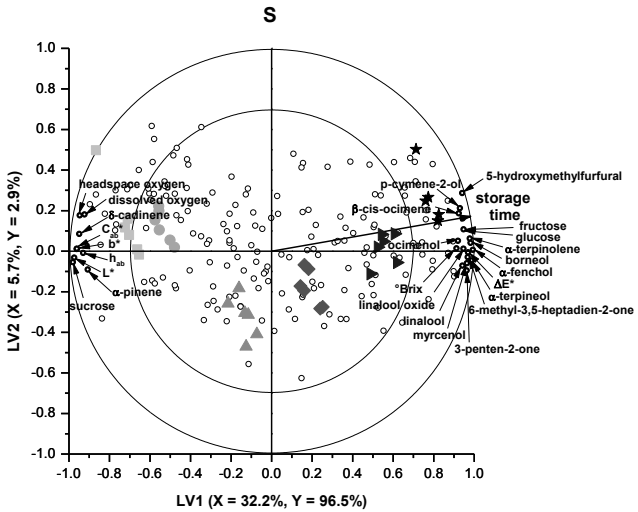


Figure 2. PLS biplot visualising the effect of storage time on the mango juice quality parameters (targeted) and the volatile fraction (untargeted) at 42 °C. Samples are represented by differently shaped symbols; storage time is indicated by colour scale from grey to black. The open circles show different parameters and volatiles of which the ones with absolute VID values higher than 0.80 are identified and marked in bold. Positive VID coefficients represent an increase in value or concentration during storage while negative coefficients indicate a decrease. Inner and outer ellipses represent correlation coefficients of 70 and 100%. The vectors represent the correlation loading for the continuous *Y*-variable (storage time). The percentages of the variances in *X* and *Y* explained by each latent variable (LV1 and LV2) are indicated on the respective axes.


Control
LV1 (X = 30.7%, Y = 93.5%)
LV2 (X = 10.3%, Y = 4.7%)
isoborneol
p-cymene-2-ol
5-hydroxymethylfurfural
p-cymene-8-ol
storage time
headspace oxygen
dissolved oxygen
linalool
borneol
α-fenchol
glucose
myrcenol
ocimenol
α-terpineol
linalool oxide
ΔE*
3-penten-2-one
fructose
α-terpinene
sucrose
AA250
LV1 (X = 30.3%, Y = 97.8%)
LV2 (X = 4.4%, Y = 2.0%)
headspace oxygen
5-hydroxymethylfurfural
isoborneol
furfural
storage time
fructose
glucose
ocimenol
linalool oxide
ΔE*
δ-cadinene
myrcenol
borneol
ascorbic acid
camphene
α-fenchol
α-pinene
3-penten-2-one
α-terpinolene
sucrose
linalool
α-terpineol
δ-selinene
AA500
LV1 (X = 32.2%, Y = 96.0%)
LV2 (X = 6.1%, Y = 3.1%)
isoborneol
5-hydroxymethylfurfural
3-penten-2-one
linalool oxide
furfural
storage time
δ-cadinene
ocimenol
ascorbic acid
glucose
borneol
camphene
fructose
α-pinene
α-terpinolene
linalool
sucrose
α-fenchol
α-terpineol
α-terpinene
CA
LV1 (X = 35.3%, Y = 58.6%)
LV2 (X = 12.51%, Y = 33.8%)
ocimene quintoxide
linaloyl oxide
sucrose
γ-terpineol
dissolved oxygen
5-hydroxymethylfurfural
headspace oxygen
1,4-cineole
ethyl acetate
β-terpineol
storage time
ethyl butanoate
α-myrcene
p-cymene-8-ol
β-caryophyllene
β-cis-ocimene
1,8-cineole
isoborneol
butyl butanoate
dimethyl sulphide
linalool oxide
°Brix
ocimenol
ΔE*
myrcenol
p-cymene
3-penten-2-one
glucose
CA+AA250
LV1 (X = 53.4%, Y = 67.9%)
LV2 (X = 17.5%, Y = 26.9%)
ocimene quintoxide
linaloyl oxide
α-pinene
5-hydroxymethylfurfural
sucrose
γ-terpineol
ethyl acetate
furfural
α-myrcene
storage time
ethyl butanoate
p-cymene-8-ol
ascorbic acid
ΔE*
1,8-cineole
ocimenol
α-terpinene
isoborneol
linalool oxide
a*
butanoic acid
myrcenol
°Brix
CA+AA500
LV1 (X = 33.0%, Y = 95.5%)
LV2 (X = 8.3%, Y = 3.9%)
caryophyllan-2,6-β-oxide
γ-terpineol
ocimene quintoxide
linaloyl oxide
1,4-cineole
5-hydroxymethylfurfural
furfural
α-pinene
storage time
sucrose
α-myrcene
1,8-cineole
ethyl acetate
p-cymene-8-ol
isoborneol
ocimenol
linalool oxide
a*
ΔE*
myrcenol
butanoic acid
α-terpinene



Storage time

week 0 week 1 week 2 week 4 week 6 week 8

Figure 3. PLS biplot visualising the effect of storage time on the mango juice quality parameters (targeted) and the volatile fraction (untargeted), per formulation. Samples were stored at 42 °C for 8 weeks: Control (mango puree and water), control with addition of ascorbic acid up to 250 mg L^{-1} (AA_{250}), control with addition of ascorbic acid up to 500 mg L^{-1} (AA_{500}), control with addition of citric acid (CA), control with addition of citric acid and ascorbic acid up to 250 mg L^{-1} ($CA+AA_{250}$), control with addition of citric acid and ascorbic acid up to 500 mg L^{-1} ($CA+AA_{500}$) and control with addition of sugars (S). Stored samples are represented by differently shaped symbols. The open circles represent different quality parameters (targeted) and volatiles (untargeted), of which only the ones selected through the VID procedure are identified and marked in bold (**Table 1**). Inner and outer ellipses represent correlation coefficients of 70 and 100%. The vectors represent the correlation loading for the continuous *Y*-variable (storage time). The percentages of the variances in *X* and *Y* explained by each latent variable (LV1 and LV2) are indicated on the respective axes.

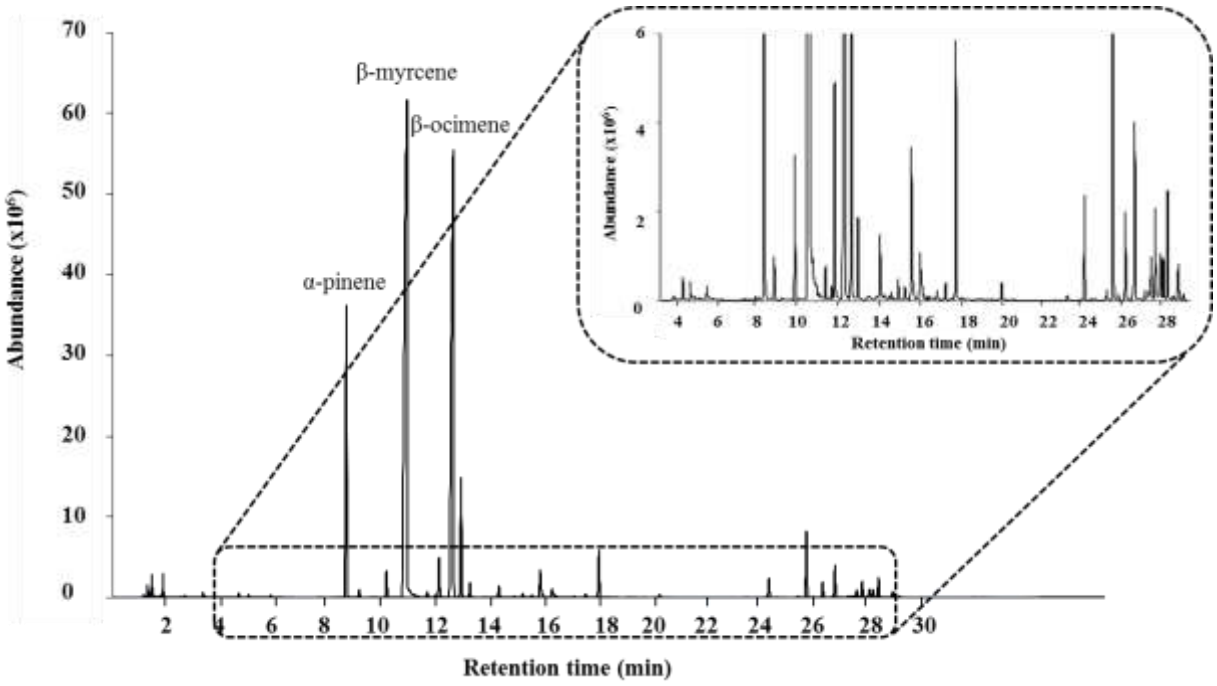


Figure 4. Total ion chromatogram of the headspace of control pasteurised mango juice at the beginning of storage (week 0), obtained by the selected headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting method.